

INFLUENCE OF ASSOCIATION ON THE SEXUAL PHASES OF GASTROPODS HAVING PROTANDRIC CONSECUTIVE SEXUALITY

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The studies of Orton (1909, 1912, 1922), Kleinstaubner (1913), Giese (1915), Gould (1917*a*, 1917*b*, 1919), Coe (1935, 1936, 1938*a*, 1938*b*), Ishiki (1936) and others have shown the occurrence of protandric consecutive sexuality in all members of the pectinibranch family Calyptraeidae (or Capulidae) which have been investigated hitherto, making it appear probable that this type of sexuality is characteristic of the entire family.

The conditions which inaugurate the change from male to female phase, however, have not yet been fully elucidated. Moreover, Gould (1919) concluded that the development of the functional male phase in *Crepidula plana* depended upon the secretion of a masculinizing substance by an older individual with which the sexually immature or "neuter" individual was associated. Actual contact was deemed unnecessary, since it was assumed that the secretion could pass through the water. This conclusion has been widely quoted in the textbooks, although it has been shown recently (Coe, 1935, 1936) that solitary or isolated young of that species may under favorable conditions assume the functional male phase when living entirely isolated from other individuals. Nevertheless, the masculine characteristics when already established may be accentuated and prolonged in that and other species by the association of the male with a female.

Ishiki (1936) concluded that in both *Crepidula walshi* and *C. aculeata* some stimulation of a chemical nature "is carried toward the small individual from the female" which prolongs the male phase as long as association with the female continues. The present writer (1938*a*), on the other hand, discredits the idea of a masculinizing hormone passing from the female to the associated male and assumes that the accentuation and prolongation of his masculine characteristics result from hormones secreted within his own body as the result of stimuli received by his nervous system from the body of the female through the sense

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organs in his penis and tentacles. Such sensory stimuli doubtless dominate his autonomic responses during copulation as well.

It has been shown (Coe, 1936) that the three common species of *Crepidula* (*C. fornicata*, *C. convexa* and *C. plana*) on the Atlantic coast respond in a somewhat similar manner to the influences of association, although the individuals of the three species differ so greatly in their habits. It has seemed desirable, therefore, to extend these studies to other species in order to determine more fully, if possible, the essential character of the responses observed in nature and experimentally.

There are some ten or eleven valid species of the genus *Crepidula* reported from the Pacific coast. Four of these (*C. onyx*, *C. lingulata*, *C. adunca* and *C. nummaria*) form the basis for the investigation here

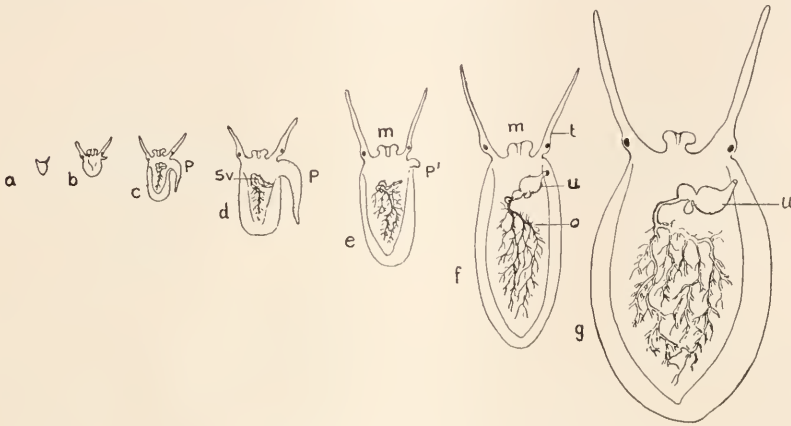


FIG. 1. Successive sexual phases in *Crepidula nummaria*, showing the development and absorption of the penis and the reorganization of the reproductive system preceding the female phase; a, recently hatched young; b, early male phase, with primordium of penis behind right tentacle; c, young functional male; d, mated male; e, transition stage; f, female phase, dwarf form; g, female when growing on unrestricted surface; m, mouth; o, ovary; p, penis; sv, seminal vesicle; u, uterus and seminal receptacle; t, tentacle.

reported. Each of these species can be obtained in abundance for experimental studies at all seasons of the year. All of them agree in having protandric consecutive sexuality, but each species differs in the associative behavior of the two sexual phases and in their responses to experimental conditions in the aquarium.

In these species, as in others previously studied (Gould, 1917a, 1917b; Coe, 1935, 1936, 1938a; Ishiki, 1936) the male phase becomes functional at an early age, when the body has reached only a small fraction of its definitive size (Fig. 1). After spermatogenesis has commenced a long, muscular penis grows out from the neck behind the right tentacle (Fig. 1). By means of this organ the sperm which have

been stored in the seminal vesicles are transferred as opportunities permit to the uterus and seminal receptacle of an older individual in the female phase (Fig. 1).

During the transition from the male to the female phase the residual spermatid cells are cytolized and such small ovocytes as may have become differentiated, but not cytolized, during the process of spermatogenesis increase rapidly in size and soon begin the deposition of yolk. The gonad thereby changes from a spermary to an ovary. Sometimes this change takes place rapidly but often there is a prolonged period of apparent inactivity, during which the gonadal follicles contain only relatively few ovogonia. In the latter case the body may grow rapidly while the genital duct is being transformed into an oviduct by the absorption of the seminal vesicles and the terminal portion enlarged and extended to form the uterus and seminal receptacle (Fig. 1). Under favorable conditions ovogenesis then follows rapidly.

Accompanying the morphological changes from male to female phase are fundamental alterations of an appropriate nature in the individual's instinctive behavior. The activities characteristic of the male are supplanted by such as are concerned with the formation and attachment of the egg capsules and the provisions for the protection and oxygenation of the developing larvae.

Unfavorable conditions, such as forced movements, accumulation of waste materials, lack of food or of oxygen, abnormal temperature or parasitism, when not fatal, terminate the male phase without the compensatory development of the female characteristics, leading to an essentially neutral sexual condition. If a favorable environment is restored the transformation to the female phase is resumed, but not otherwise. Occasionally the male phase may be aborted and the female functional. Excessive infestation by trematode larvae may also result in the almost complete destruction of the gonad after the female phase has become established.

Since only fragmentary accounts of the biology of these species have been published it seems advisable to discuss each of the four species separately, particularly in regard to the sexual phases and reproductive behavior.

CREPIDULA ONYX SOWERBY

(*C. rugosa* Nuttall)

This species closely resembles *C. fornicata* of the Atlantic coast—but now introduced and established on the Pacific coast—both in appearance and in habits. Pyramidal groups of three to sixteen or more individuals are often found in muddy bays. In such groups the basal individual is attached to an empty shell of the same or of some other

species of mollusk. This and one or more of the superimposed individuals are usually in the female phase, while transition forms, sedentary males, supplementary males and sexually immature individuals may be superimposed in the sequence named (Fig. 2, *a*).

The supplementary males move about from one female to another, while the sedentary males retain the same position permanently with respect to the immediately underlying individuals. Nevertheless, these sedentary males are capable of sufficient movement to enable them to fertilize females not immediately adjacent. They return to their original positions after copulation has been accomplished or at the approach of daylight, for the crepidulas are largely nocturnal in their activities.

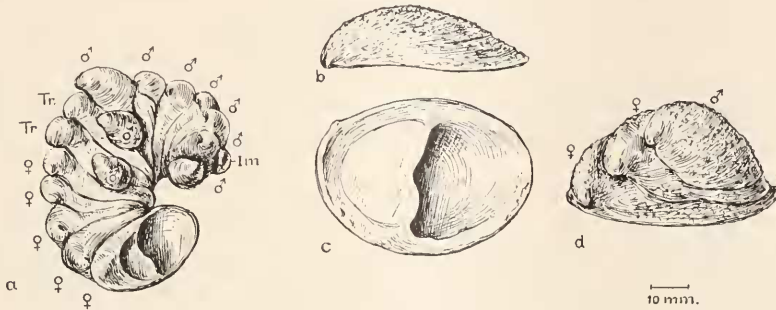


FIG. 2. *Crepidula onyx*; *a*, typical form, large group consisting of seventeen individuals from intertidal mud flat; the group includes five basal females, two transition individuals and seven superimposed functional males, with two small supplementary males and one sexually immature young (*Im*); *b*, large, isolated female of the roughened, flattened form (*rugosa*); *c*, same from ventral surface of empty shell; *d*, *rugosa* form, small group consisting of two basal females and one superimposed male. Scale indicates natural size.

Before beginning transition to the female phase the sedentary males often reach a length almost equal to that of some of the underlying females. In such males the transition period is very brief (Fig. 6).

The supplementary and solitary males, on the other hand, as is also the case with many mated males that are isolated experimentally when of small size, have a much longer transition period. During this period they grow considerably but not sufficiently rapidly to equal the sedentary males in size (Fig. 6). Growth also continues after the female phase is reached.

Another habitat form has a flat, oval shell and has been given the varietal name *rugosa* because of the roughened surface (Fig. 2, *b*, *c*, *d*). Individuals of this form occur singly or in groups of two, three or four, usually attached to *Pecten* or other large shells, or to rocks or spiles. These often become much larger than those living on muddy bottoms.

Females may exceed 60 mm. in length, while in mated or associated individuals the male phase may continue until the shell has a length of 35 mm. or more. A large proportion of the males, however, are solitary and transform to the female phase when relatively small. In other habitats only dwarf individuals are found.

In this species the accentuation and prolongation of the male phase in associated or mated individuals as compared with those which remain supplementary or solitary either by chance or by choice is very great (Fig. 6). Individuals of all ages are remarkably hardy, living for many months in the aquarium if sufficient food is available. Isolation of mated males usually, but not in all cases, terminates the male phase and inaugurates the female phase promptly.

CREPIDULA LINGULATA GOULD

(*C. dorsata* Broderick)

This species likewise occurs in several different forms as the result of various environmental conditions.

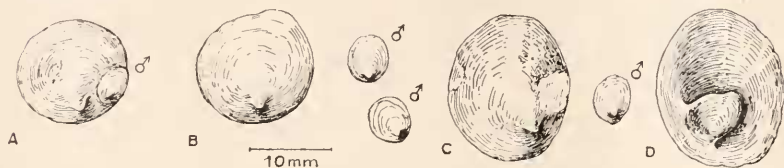


FIG. 3. *Crepidula lingulata*. A, mated pair. B, female with two not closely associated males. C, female with mated male removed, showing "scar" indicating that the male may sometimes remain attached for a considerable period. D, shell of female from ventral side to show character of internal partition.

The male phase commonly becomes functional when the young individual has reached a length of 3 to 5 mm., while the transition stages occur at about 8 to 11 mm. The average individual assumes the female phase when about 9 to 12 mm. long, after which growth usually continues until a length of 18 to 20 mm. is reached. An occasional female may be more than 25 mm. long.

It would appear that only temporary matings are the rule in this species since not more than 5 per cent of the mature females observed were accompanied by mated males (Fig. 3) at the time of collection. Only about 5 per cent of the other females showed scars to indicate where the males had been formerly attached (Fig. 3, c). All of them, however, had sperm in their seminal receptacles, indicating that temporary matings, presumably nocturnal, had taken place. The males were scattered about in the neighborhood but only occasionally in the immediate vicinity of the females. Consequently in this species no

evidence is available as to the effects of association on the sexuality of the male. None of the functional males observed exceeded 12 mm. in length.

CREPIDULA NUMMARIA GOULD

(*C. nivea* C. B. Adams)

This species also is very variable in form as a response to the habitat of the individual. When growing within the narrow space inside an empty shell of *Tegula* or other small univalve, the adults in the female phase are very slender and only 14 to 16 mm. in length (Fig. 4, *a, b*). When attached to the surface of a large shell, stone or other solid object, however, the shell becomes broadly oval (Fig. 4, *c, d*) or even nearly circular, as was the case with Gould's type specimen, and may sometimes exceed 25 mm. in length.

There can be no further doubt as to the specific identity of the two forms, since the newly hatched young of the slender form have grown into the broad form *nivea* when reared in the aquarium at the Scripps Institution of Oceanography.

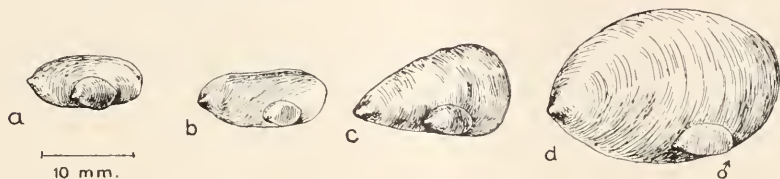


FIG. 4. *Crepidula nummaria*. *a, b*, mature females, dwarf form, with mated males, living in small univalve shells; *c, d*, same when living on surface which allows unlimited space for growth. Scale shows natural size.

The young often reach the functional male phase within a few weeks after hatching, when the body is less than 3 mm. in length (Figs. 4, 6). Others remain sexually immature until they are 6 to 8 mm. long. The sexual transformation commonly takes place at a length of 7 to 10 mm.; most individuals more than 10 mm. long are in the female phase (Fig. 6). The successive stages in the development and transformation of the reproductive system in the two sexual phases are shown in Fig. 1. The early gonad is less distinctly ambisexual than in the other species investigated, since very few ovocytes become differentiated until toward the end of the transition period.

The males are not usually found in the mating position on the females (Fig. 3) but remain associated nearby or in contact until afforded opportunities for copulation. The influence of association in the accentuation and prolongation of the masculine characteristics becomes evident when series of associated and solitary males are com-

pared (Fig. 6). Unfavorable conditions postpone sexual maturity as well as terminate the male phase and delay the differentiation of the female phase.

CREPIDULA ADUNCA SOWERBY

This species resembles *C. convexa* of the Atlantic coast (but now established on the Pacific coast) in its habits (Coe, 1936).

Conklin (1897, 1898) studied the reproduction and development of this species and Moritz (1938) has described the anatomy of the young male. The mated males retain the mating position for a long period (Fig. 5) but usually leave the female at the beginning of the transition phase.

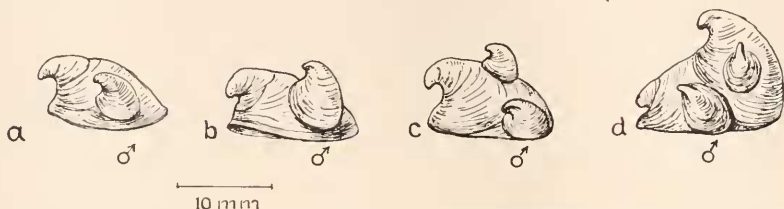


FIG. 5. *Crepidula adunca*. Outlines showing mated females, with associated males; (a) female with male of average size; (b) female with relatively large male; (c) female with mated male and supplementary male; (d) two superimposed females each with mated male.

The young may reach the functional male phase within a few weeks after hatching, sometimes when less than 4 mm. in length. The sexual transformation commonly begins when a length of 9 to 10 mm. is reached (Fig. 6). The influence of association or mating on the accentuation and prolongation of the male phase is less evident in this species than in most others that have been studied, although the tabulation of many observations shows a significant difference between solitary and mated individuals (Fig. 6).

COMPARISON WITH OTHER SPECIES

Three of these species, *C. onyx*, *C. nummaria* and *C. adunca*, demonstrate the influence of association on the accentuation and prolongation of the male phase (Fig. 6). In the fourth species, *C. lingulata*, no such evidence has been obtained. In none of these species, however, nor in any member of the family Calyptraeidae thus far investigated, is such association a prerequisite for the functional development of the male phase, but only for its accentuation and prolongation.

The three species of *Crepidula* on the Atlantic coast, *C. convexa*, *C. plana* and *C. fornicata*, show the same influence of association as do

the three Pacific coast species mentioned (Coe, 1936, 1938a). This is likewise true of the two Japanese species studied by Ishiki (1936) as well as of *Crucibulum spinosum* (Coe, 1938b).

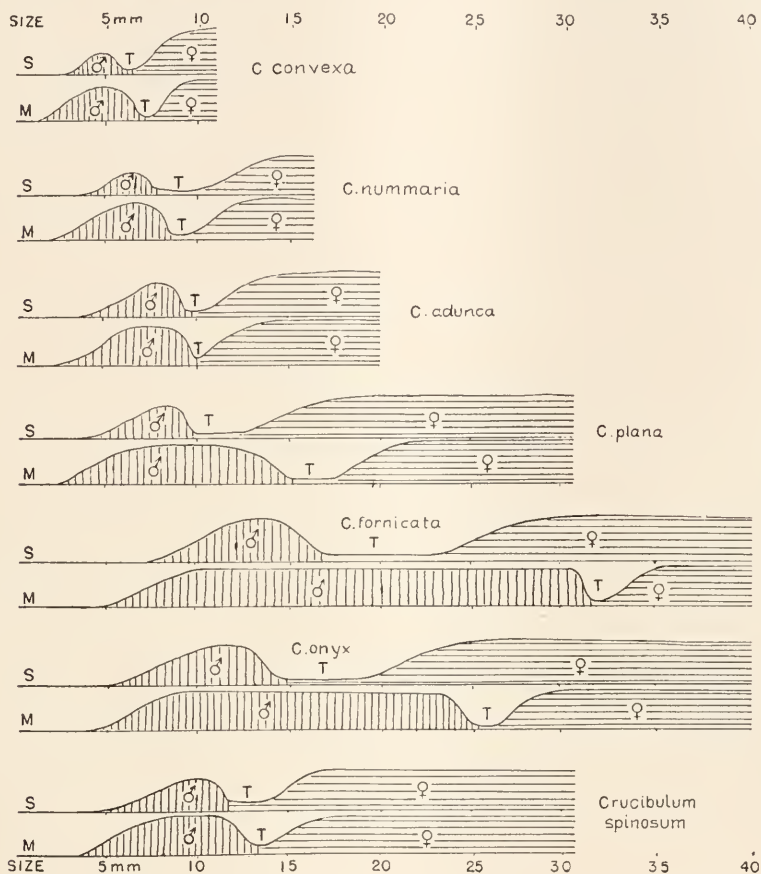


FIG. 6. Diagrams indicating the relative duration of each of the sexual phases in isolated or solitary males (S) as compared with associated or mated males (M) in six species of *Crepidula* and in *Crucibulum spinosum*. In each case the height of the curve indicates the relative development of the reproductive system. The sizes given indicate the lengths of the shells. For each species it will be noted that the solitary individuals have a briefer functional male phase and a longer transition period (T) than the corresponding associated individuals. All the diagrams are drawn at the same horizontal scale, although the heights of the curves are not strictly relative for the different species.

The comparative development of the gonads in the sexually functional and transition phases in solitary and mated or associated males in six species of *Crepidula* and in *Crucibulum spinosum* is shown diagram-

matically in Fig. 6. All are drawn at approximately the same scale as regards body length. The accentuation and prolongation of the functional male phase in associated or mated (*M*) as compared with isolated or solitary (*S*) individuals are characteristic for each species. The transition period is longer in solitary individuals.

It will be noted also that the male phase is inaugurated at a smaller size and a presumably younger age in mated than in solitary individuals. It has been assumed that the mated young develop functional maleness earlier as a result of their association but it seems more plausible that those young which reach the male phase earliest are the ones that are most likely to be mated earliest by virtue of the early development of their masculine instincts which lead them to seek the female. Those with less masculine characteristics are presumably more likely to remain solitary (Coe, 1938a). Indeed, it seems not improbable that there may also be minor genetic differences in the two groups.

Whether the mating is to be (a) permanent, as is the case with some individuals in *C. onyx* and *C. fornicata* or (b) long-continued, as may occur in *C. convexa*, *C. adunca* and *Crucibulum spinosum*, or (c) highly variable in length, as in *C. plana* and *C. nummaria*, or (d) comparatively brief, as in *C. lingulata*, is also presumably dependent upon genetic differences. Both specific differences and individual peculiarities in instinctive behavior are doubtless involved, as well as environmental influences. Also the question of chance as distinguished from choice must be taken into consideration before a conclusion is reached as to the actual significance of the behavior observed.

With these precautions in mind the nature of the influence whereby the presence of the female accentuates and prolongs the functional male phase may be considered. Experimental evidence indicates that the empty shell of the female does not have the same effect (Coe, 1938a). That a masculinizing hormone or other chemical substance passes through the water from the older to the younger individual or from the female to the associated male, as Gould (1917b, 1919) and Ishiki (1936) have supposed, seems improbable for the reasons stated in the introduction to this article.

There can be no doubt, however, that the associated male receives stimuli from the female through his sense organs in the penis and tentacles. These sensory stimuli not only affect the behavioristic instincts of the male but they also elicit responses at the time of copulation, resulting in the transfer of sperm to the seminal receptacle of the female. They doubtless also influence such other parts of the male's autonomic system as are concerned with the accentuation and pro-

longation of his masculine characteristics, presumably through the secretion of hormones within his body.

In the immature male the activation of the spermary and the inauguration of spermatogenesis precede the growth of the penis. Moreover, cessation of active spermatogenesis precedes the absorption of the penis at the termination of the male phase whether such termination be due to isolation, to unfavorable environment or to the internal changes which take place when a certain degree of maturity is reached. Sexual metamorphosis in these mollusks resembles other types of metamorphosis in animals in that the juvenile (male) phase can be abbreviated or prolonged experimentally but the sequence of the sexual phases cannot be reversed. It may be assumed that all these changes are mediated by internal secretions.

It is significant that in these species of *Crepidula* and *Crucibulum*, as well as in other described cases of protandric consecutive sexuality in planarians, nemerteans, oligochaetes, chaetopods, echinoderms, crustacea, pulmonate gastropods, oysters, clams, teredos and other forms the activation of the male constituents of the primary ambisexual gonad always precedes the development of such secondary morphological and behavioristic sexual characteristics as are peculiar to the functional male phase. The external environment in many cases determines the extent of this phase. The termination of the male phase and the metamorphosis to the functional female phase are similarly correlated with the activation of the ovarian constituents of the gonad. Such also occurs in the cases of alternative sexuality, as in the oviparous oysters, and of rhythmical sexuality, as in the larviparous oysters. That these developmental and transformational activities are mediated by the secretion of growth-stimulating substances to which certain localized groups of cells are attuned seems highly probable.

SUMMARY

1. The functional sexual phases of four species of Pacific coast gastropods with protandric consecutive sexuality have been investigated with regard to the conditions which control the change of sexual phase and particularly with respect to the influence of association or mating on the individual's sexuality.

2. In none of these species nor in six species previously studied is association a prerequisite for the development of the functional male phase, although isolation of these males after a prolonged mating period usually terminates the male phase promptly.

3. In nine of the ten species studied intimate association or permanent mating of the young male with one or more individuals in the female phase tends to accentuate the masculine characteristics and to postpone sexual transformation by prolonging the male phase. In five of these ten species the influence of association is very conspicuous; in four it is apparent but not conspicuous; in only one is the evidence inconclusive.

4. There seems to be no evidence that the accentuation and prolongation of the male phase by association is due to any masculinizing chemical secretion or hormone which passes through the water from the body of the female to that of the male, as some investigators have assumed; it seems more probable that stimuli received by the male through the sense organs in his penis and tentacles during association with the female are of such a nature as to influence his masculine characteristics, presumably by the mediation of hormones secreted within his own body.

5. Sexual maturity and sexual metamorphosis in these mollusks, like other types of metamorphosis in animals, are subject to environmental influences and can be accelerated, postponed or inhibited experimentally, but the sequence of sexual phases cannot be reversed.

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